The Egyptian Journal of Medical Human Genetics (2014) 15, 369–377

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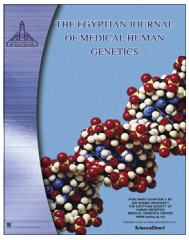

ORIGINAL ARTICLE

Subclinical renal abnormalities in young thalassemia major and intermedia patients and its relation to chelation therapy

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Received 28 May 2014; accepted 25 June 2014
Available online 11 August 2014

KEYWORDS

Glomerular;
Tubular;
Beta-thalassemia;
β2 microglobulin;
Chelation therapy

Abstract *Background:* Limited data are available about renal involvement in thalassemia patients. Renal dysfunction in these patients seems to be multifactorial attributed mainly to long standing anemia, chronic hypoxia, iron overload and toxicity of iron chelators.

Objective: To assess the frequency of subclinical glomerular and tubular dysfunctions in children and adolescents with β-thalassemia major and intermedia, and to correlate these findings with the degree of iron overload and type of chelation therapy.

Methods: The study included 40 thalassemia major and 20 thalassemia intermedia pediatric patients recruited from the Pediatric hematology clinic, Ain Shams University. Serum sodium, potassium, phosphorous and creatinine, and urinary sodium, potassium, phosphorous, protein/creatinine ratio and urinary β2 microglobulin were measured. Fractional excretion of sodium and potassium was calculated.

Results: The mean level of serum creatinine in all patients was within the normal range and comparable in both TM and TI groups (0.17 ± 0.06 and 0.18 ± 0.07 mg/dl, respectively, $P > 0.05$). The mean eGFR was higher than normal range in both TM and TI groups (552.65 ± 231.73 and 472.15 ± 272.99 ml/min, respectively). Mean level of urinary β2 microglobulin was within the normal range (0.13 ± 0.05 and 0.10 ± 0.03 μg/ml) in TM and TI patients, however, it was significantly higher in TM patients ($P = 0.009$). Urinary β2 microglobulin was positively correlated to

Abbreviations: A/C, albumin/creatinine ratio; β2MG, urinary β2 microglobulin; CBC, complete blood count; FENa, fractional excretion of sodium; FEK, fractional excretion of potassium; Hb, hemoglobin; HPLC, high-performance liquid chromatography; NAG, *N*-acetyl-β-d-glucosaminidase; TM, thalassemia major; TI, thalassemia intermedia; U pr/Cr ratio, urinary protein/creatinine ratio

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Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2014.06.004

both transfusion index and serum ferritin level ($P < 0.05$). Tubular reabsorption of phosphorus (TRP) was significantly higher among TM patients ($P = 0.037$). The mean height and height percentile were lower in the poorly chelated group (serum ferritin $\geqslant$2500 ng/ml) than the well chelated group. In addition, the mean serum sodium and urinary protein/creatinine ratio were significantly higher in the poorly chelated group ($P < 0.05$).

Conclusion: Subclinical renal affection can start earlier in TM patients compared to TI. Poor chelation is associated with early signs of renal affection. Periodic renal assessment of those patients is mandatory as they may be affected by hidden renal dysfunction.

1. Introduction

β-Thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the β chains of hemoglobin (Hb) resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals [1]. β-Thalassemia intermedia (β-TI) encompasses a wide clinical spectrum of the β-thalassemia (β-thal) phenotype. Some β-TI patients are asymptomatic until adult life, whereas others are symptomatic from as young as 2 years of age [2]. The severity of β-TI depends on the degree of imbalance between α and non-α chains as well as other genetic and environmental factors that modify the natural history of the disease [3]. β-Thalassemia major (β-TM) is an inherited Hb disorder characterized by chronic anemia and iron overload due to transfusion therapy and gastrointestinal absorption. Iron overload causes most of the associated mortality and morbidity [1,4]. A number of clinical complications are commonly associated with β-TI, affecting the lives of patients. Prevention of these complications is ideal since they may be difficult to manage [5,6]. The cost of providing lifelong medical care to patients with thalassemia according to the standards adopted in the developed countries is extremely high. The burden of thalassemia imposed on the health systems of developing countries is unbearable [7].

With increased duration of survival of children with β-thalassemia major, the effects of iron overload in the liver, pancreas, and heart become more severe, however renal involvement has received little attention [8]. Renal dysfunction may occur in β-thalassemia major patients showing no clinical symptoms and before the manifestations of any other complications [9]. Renal dysfunction in these patients is not known well and seems to be multifactorial; attributed mainly to long-standing anemia, chronic hypoxia, iron overload and toxicity of iron chelators [10].

1.1. Objective

This study aimed to investigate the frequency of subclinical glomerular and tubular dysfunctions in children and adolescents with β-thalassemia major and β-thalassemia intermedia, and to correlate these findings with the degree of iron overload and type of chelation therapy.

2. Patients and methods

This cross sectional study included 60 patients recruited from the regular attendants of the Pediatric Hematology Clinic, Pediatric Hospital, Ain Shams University. Patients were divided into two groups; Group 1 consisted of 40 patients with β thalassemia major (18 males and 22 females) with age ranging from 2.5 to 18 years and with a mean age of 10.78 ± 4.03 years, and Group 2 consisted of 20 patients with β thalassemia intermedia (11 males and 9 females) with age ranging from 2.5 to 15 years and with a mean age of 8.78 ± 3.45 years. Group 1 patients were further subdivided into 2 groups according to the serum ferritin level: Well chelated group (with serum ferritin $<$2500 ng/ml) that included 30 patients, 14 males and 16 females, with age ranging from 3 to 18 years and mean age of 11.35 ± 4.25 years. Poorly chelated group (with serum ferritin $\geqslant$2500 ng/ml) that included 10 patients, 4 males and 6 females with age ranging from 2.5 to 12 years and a mean age of 9.1 ± 2.8 years. The procedures applied in this study were approved by the parents of children as well as approved by the Ethics Committee of Human Experimentation of Ain Shams University, and are in accordance with the Helsinki Declaration of 1975.

2.1. Diagnostic criteria of thalassemia patients

Diagnosis of each type of thalassemia was based on age at presentation, markers of chronic hemolysis as well as qualitative and quantitative analysis of Hb. The studied β-TI patients had the following criteria at the time of initial diagnosis; age at presentation was more than 2 years, mean Hb level of 8–10 g/dl, HbF $<$ 50% and HbA2 $>$ 4%. The criteria of β-TM patients at the time of initial diagnosis were: age at presentation was less than 2 years, mean Hb level of 6–7 g/dl, HbF $>$ 50% and HbA2 $<$ 4% [11]. Exclusion criteria for patients under this study included other hemoglobinopathies (thalassemia minor or sickle-thalassemia), any associated hemolytic disorder (e.g., glucose 6-phosphate dehydrogenase deficiency), those with urinary tract infection at time of sampling and patients with serum creatinine above the upper normal limit for age.

The main indications for splenectomy in the studied thalassemia patients were hypersplenism, splenic pain, leucopenia and thrombocytopenia, growth retardation, severe exercise intolerance, increased transfusion demand or symptomatic splenomegaly [12]. For transfusion status, transfusion therapy was initiated in β-TI patients mainly for failure to thrive in childhood, bone deformities, progressive splenic enlargement, persistent worsening anemia, or development of complications during the course of the disease. β-Thalassemia major patients were transfused on regular interval transfusion protocols based on Hb level (once every 2–5 weeks for a pre transfusion Hb of $<$7.0 g/dl). Iron chelation therapy was administered for at

least 1 year or else the patient was considered not to be chelated.

3. Methods

All the included patients were subjected to full medical history taking including age at examination, age at first transfusion, type of chelator, duration of chelation, compliance to chelation therapy, transfusion index, mean pretransfusion hemoglobin, serum ferritin, platelet count, liver functions, presence or absence of hepatitis and symptoms suggestive of diabetes mellitus. A thorough clinical examination was performed for all patients with stress on anthropometric measures, pubertal stage by Tanner's classification, clinical evidence of complications of iron overload, and skin hemosiderosis.

3.1. Sample collection

Peripheral blood samples were collected on ethylene diamine tetra-acetic acid (EDTA) (1.2 mg/ml) for complete blood count (CBC) and hemoglobin analysis by high-performance liquid chromatography (HPLC). For chemical analysis, clotted samples were obtained, and serum was separated by centrifugation for 15 min.

3.2. Diagnostic testing

Laboratory investigations included CBC using SysmexXT-1800i (Sysmex, Japan), qualitative and quantitative hemoglobin analysis using HPLC by D-10 (BioRad, Marnes La Coquette, France), liver and kidney function tests as well as serum ferritin on Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). Serum ferritin level was measured routinely every 3 months during the study with calculation of the mean value of the last year prior to the study to know the ferritin trend. Markers of hepatitis virus B and C were assessed by enzyme-linked immunosorbent assay (ELISA). We assessed different parameters of kidney function for all patients including serum electrolytes (sodium, potassium and phosphorous), serum creatinine, urinary electrolytes (sodium, potassium and phosphorous), urinary protein/creatinine ratio, estimated glomerular filtration rate (eGFR) by Schwartz equation for children: eGFR (ml/min/1.73 m^2) = height (cm) × constant/serum creatinine (mg/dl), where height was expressed in "cm" and constants are 0.44 (for children <2 years) and 0.55 (for children $\geqslant 2$ years) [13], fractional excretion of sodium (FENa%), fractional excretion of potassium (FEK%) and urinary β2-microglobulin by ELISA (supplied by ORGENTEC). For measurement of urinary electrolytes, five milliliters of urine was divided into two aliquots; one for the immediate assessment of sodium, potassium, phosphorous, creatinine and protein and the other aliquot was stored at −20 °C for the subsequent assay of β2-microglobulin.

3.3. Statistical method

Statistical analysis was performed using SPSS 17.0 statistical package. All results were expressed as mean and SD values for parametric data and median, IQR for non-parametric quantitative data. Student's *t*-test was used for mean values, Mann–Whitney test for non-parametric quantitative data and Chi-square test for comparing categorical variables. Pearson correlation and Spearman's rho were used for correlation in parametric and non-parametric data respectively. P value <0.05 was considered significant.

4. Results

4.1. Clinical and demographic data of the studied patients

Our study included 60 thalassemic patients, 40 (66.7%) patients with TM and 20 (33.3%) patients with thalassemia intermedia. The demographic characteristics of our patients are shown in Table 1. Age at first transfusion was significantly lower in TM patients compared to TI patients ($P < 0.001$). As regards weight, height and BMI percentiles, they were comparable among TM and TI patients.

50% of TI patients were not on iron chelation therapy compared to only 5% in TM patients ($P < 0.0001$). Deferiprone was the most prevalent iron chelator. It was used as a single chelator by 27.5% of TM patients and 25% of TI patients. 17.5% of TM patients and 15% of TI patients were treated by deferoxamine only. 25% of TM patients were on combined deferiprone and deferoxamine chelation therapy compared to only 10% 0f TI patients. The iron chelator deferasirox was used by 25% of TM patients while none of TI patients used deferasirox (Table 2). Patients with TM used a significantly higher dose of deferoxamine ($P = 0.04$) and were on longer chelation duration compared to TI patients ($P = 0.0001$). Compliance to chelation was higher in TM patients; 30 patients (75%), compared to 11 patients (55%) with TI.

Among our 60 patients, there were 22 patients in the pubertal stage; 17 (77%) of them showed delayed puberty for age, 12 patients with TM (30%) and 5 patients with TI (25%). 53% of those patients were males and 47% were females. Also, 15 (25%) of all our patients were splenectomized; 14 (35%) belonged to the TM group and only one (5%) to the TI group ($P = 0.91$) as shown in Table 3. In addition, 17 (42.5%) of our TM patients had skin hemosiderosis compared to only 2 (10%) TI patients ($P = 0.03$) (see Table 4).

In the present study, the mean height as well as the height percentile were lower in the poorly chelated group (serum ferritin $\geqslant 2500$ ng/ml) than the well chelated group (serum ferritin <2500). We found also that splenectomy cases were more prevalent in the poorly chelated group; 4 cases out of 10 (40%) compared to 10 cases out of 30 in the well chelated group (33.3%), (Results not shown). In addition, the mean serum sodium and urinary protein/creatinine ratio were significantly higher in the poorly chelated group than in the well chelated group ($P < 0.05$) as shown in Table 5.

4.2. Laboratory parameters of the studied patients

Laboratory serum and urine parameters of both thalassemia major and intermedia are shown in Table 4. The mean level of serum creatinine in all our cases was within the normal range and comparable in both TM and TI groups ($P > 0.05$). The mean estimated GFR (done by Schwartz equation) among our studied patients was higher than the normal range in both TM and TI groups indicating glomerular hyperfiltration. Also, the mean urinary protein/creatinine ratio

Table 1 Comparison of demographic data among TM and TI patients.

	TM N = (40 cases)	TI (N = 20 cases)	Test	P
Gender			$X^b/z^c/t^d$	
Male N (%)	18 (45%)	11 (55%)	0.53[b]	0.47
Female N(%)	22 (55%)	9 (45%)		
	Mean ± SD	Mean ± SD	Test	P
Age (years)	10.78 ± 4.03	8.78 ± 3.46	1.91[d]	0.06
Age at 1st transfusion (months)	14.60 ± 9.23	45.95 ± 9.23	−4.45[c]	**0.0001**[a]
Weight (kg)	30.56 ± 11.49	26.43 ± 10.84	−1.49[c]	0.14
Height (cm)	130.93 ± 17.98	122.8 ± 21.64	2.30[d]	0.058
BMI (kg/m^2)	17.22 ± 2.65	18.37 ± 4.26	−0.68[c]	0.49
Weight percentile			19.62[b]	**0.02**[a]
Below 10th	17 (2.5%)	12 (60%)		
Above 10th	23 (97.5.5%)	8 (40%)		
Height percentile			3.50	0.94
Below 10th	23 (57.5%)	15 (75%)		
Above 10th	17 (42.5%)	5 (25%)		
BMI percentile			11.06[b]	0.35
Below 10th	13 (32.5%)	3 (15%)		
Above 10th	27 (67.5%)	17 (85%)		

TM: thalassemia major, TI: thalassemia intermedia, BMI: body mass index.
[a] Significant $p < 0.05$.
[b] Chi square test (Fisher exact).
[c] Mann–Whitney test.
[d] Independent t-test.

Table 2 Comparison of chelation use and compliance among thalassemia major and thalassemia intermedia patients.

	TM (N = 40 cases)	TI (N = 20 cases)	Test	P
Chelation type N (%)			$X^b/z^c/t^d$	
1-No chelator	2 (5%)	10 (50%)	20.081[b]	**0.0001**[a]
2-deferroxamine	7 (17.5%)	3 (15%)		
3-deferiprone	11 (27.5%)	5 (25%)		
4-deferasirox	10 (25%)	0 (0%)		
2 + 3	10 (25%)	2 (10%)		
Compliance				
Non-compliant	10 (25%)	9 (45%)	2.465[b]	0.116
Compliant	30 (75%)	11 (55%)		
Dose (mg/kg/d)	Mean ± SD	Mean ± SD		
Deferoxamine	31.76 ± 7.28	24 ± 5.48		
			2.195[d]	**0.04**[a]
Deferiprone dose	88.09 ± 12.79	82.14 ± 12.2	−1.074[c]	0.283
Duration of chelation (years)	5.41 ± 3.83	1.90 ± 2.97	−3.956[c]	**0.0001**[a]

TM: thalassemia major, TI: thalassemia intermedia.
[a] Significant $p < 0.05$.
[b] Chi square test (Fisher exact).
[c] Mann–Whitney test.
[d] Independent t-test.

in our study was higher than the normal range. We found that 11 of all our studied patients (18.3%); 8 TM patients (20%) and 3 TI (15%) patients had a protein/creatinine ratio higher than normal. The mean level of urinary β2 microglobulin in our patients was within the normal range, where only 2 TM patients (3.3%) had slightly higher levels than normal. Meanwhile, all TI patients included in the study showed normal urinary β2 microglobulin levels. However, the mean urinary β2 microglobulin was significantly lower in TI patients (P = 0.009). Normal mean levels of fractional excretion of sodium (FENa%) and fractional excretion of potassium (FEK%) were found among TM and TI patients with no significant difference between the two groups ($P > 0.05$) (see Table 6).

The mean serum potassium and serum sodium in all our cases were within the normal range but there was a significant difference between TM and TI groups, where the mean serum potassium was higher in TM patients than in TI patients (P = 0.04) and the mean serum sodium was higher among TI patients than TM patients (P = 0.01).

Table 3 Comparison of different complications among thalassemia major and thalassemia intermedia patients.

	TM	TI	Test	*P*
	(*n* = 40) *N* (%)	(*n* = 20) *N* (%)	*X*[b]	
Skin hemosiderosis	17 (42.5%)	2 (10%)	4.97	**0.03**[a]
Splenectomy	14 (35%)	1 (5%)	6.47	0.91
Liver functions(elevated liver enzymes >3 times normal IU/L)	10 (25%)	0 (0%)	5.29	**0.02**[a]
Hepatitis C +ve	4 (10%)	0 (0%)	2.14	0.14
Puberty			0.17	0.68
Not included[c]	24 (60%)	14 (70%)		
Normal	4 (10%)	1 (5%)		
Delayed	12 (30%)	5 (25%)		

TM: thalassemia major, TI: thalassemia intermedia.
[a] Significant $p < 0.05$.
[b] Fisher exact test.
[c] Prepubertal.

Table 4 Comparison of laboratory parameters among thalassemia major and thalassemia intermedia patients.

	TM *N* = 40 Mean ± SD	TI *N* = 20 Mean ± SD	Test $z^{\wedge}/t$[b]	*P*	Reference ranges
Serum K (mmol/L)	4.25 ± 0.42	4 ± 0.38	2.099[b]	**0.040**[a]	3.5–5.3 mmol/L
Serum Na (mmol/L)	132 ± 4.56	134.95 ± 2.91	−2.523[c]	**0.012**[a]	135–148 mmol/L
Serum Ph (mg/dl)	6.99 ± 2.10	5.46 ± 0.93	−3.505[c]	**0.000**[a]	Child: 4–7 mg/dl Adult: 2.5–4.5 mg/dl
Serum creatinine (mg/dl)	0.17 ± 0.06	0.18 ± 0.07	−0.852[c]	0.394	Child: 0.3–0.7 mg/dl Adolescent: 0.5–1 mg/dl
Urinary β2 microglobulin (μg/ml)	0.13 ± 0.05	0.10 ± 0.03	−2.606[c]	**0.009**[a]	0–0.3 μg/ml
Urinary Na (meq/L)	121.86 ± 81.22	117.78 ± 64.04	−0.369[c]	0.712	
Urinary K (meq/L)	71.86 ± 38.25	55.24 ± 33.43	−1.560[c]	0.119	
Urinary Ph (meq/L)	25.28 ± 13.30	24.72 ± 9.24	−0.078[c]	0.937	
Urinary creat (mg/dl)	88.88 ± 50.62	76.77 ± 31.42	−0.572[c]	0.567	
Urinary protein/creat ratio	0.27 ± 0.29	0.49 ± 1.35	−0.704[c]	0.481	Up to 0.2
FENa (%)	0.20 ± 0.20	0.28 ± 0.22	−1.216[c]	0.224	<1: prerenal failure >2: acute tubular necrosis
FEK (%)	3.51 ± 2.49	3.10 ± 1.60	−0.298[c]	0.766	<10: renal cause of hyperkalemia >10: extrarenal cause of hyperkalemia
TRP	0.99 ± 0.005	0.98 ± 0.01	−2.083[c]	**0.037**[a]	Normal >80% i.e. >.80
eGFR	552.65 ± 231.73	472.15 ± 272.99	−1.709[c]	0.087	Male: 94–140 ml/min Female: 72–110 ml/min

TM: thalassemia major, TI: thalassemia intermedia, Na: sodium, K: potassium, ph: phosphorus, creat: creatinine, Ptn: protein, FENa: fractional excretion of sodium, FEK: fractional excretion of potassium, TRP: tubular reabsorption of phosphorus, eGFR: estimated glomerular filtration rate.
[a] Significant $p < 0.05$.
[b] Independent *t*-test.
[c] Mann–Whitney test.

Table 5 Number and percentage of patients with renal dysfunction in both groups.

	Thalassemia major *N* = 40	Thalassemia intermedia *N* = 20
Abnormal GFR *n*(%)	40 (100%)	20 (100%)
Abnormal ACR *n*(%)	8 (20%)	3 (15%)
Abnormal Na	–	–
Abnormal K	–	–
Abnormal phosphorous	18 (30%)	2 (3.3%)
Abnormal β2 microglobulin	2 (3.3%)	–

GFR: glomerular filtration rate, ACR: albumin creatinine ratio, Na: sodium, K: potassium.

Table 6 Comparison of renal functions between well chelated and poorly chelated groups.

	Well chelated $N = 30$	Poorly chelated $N = 10$	Test t^b/z^c	P
Serum Na	131.22 ± 4.77	134.45 ± 2.82	2.59[b]	**0.02**[a]
Serum K	4.08 ± 0.35	4.31 ± 0.35	−1.59[b]	0.12
Serum Ph	7.29 ± 2.32	6.98 ± 0.83	−2.08[b]	0.06
Serum creatinine	0.17 ± 0.07	0.15 ± 0.05	−0.95[b]	0.36
Urinary Ptn/creat ratio(mg/mmol)	0.23 ± 0.22	0.4 ± 0.42	−1.98[c]	**0.048**[a]
Urinary β2 microglobulin	0.12 ± 0.26	0.13 ± 0.05	−0.33[c]	0.74
Urinary Na	113.94 ± 78.5	144.9 ± 89.06	−1.09[c]	0.27
Urinary K	70.35 ± 40.07	76.40 ± 33.68	−0.55[c]	0.59
Urinary P	24.21 ± 13.39	28.5 ± 13.17	−1.17[c]	0.24
Urinary creat	85.44 ± 50.85	99.2 ± 51.15	−0.91[c]	0.37
FENa	0.22 ± 0.22	0.16 ± 0.09	−0.28[c]	0.78
FEK	3.74 ± 2.75	2.83 ± 1.35	−0.59[c]	0.55
TRP	0.99 ± 0.01	0.99 ± 0.003	−0.29[c]	0.77
eGFR	558.58 ± 239.07	534.86 ± 219.23	−0.53[c]	0.60

S: serum, U: urinary, Na: sodium, K: potassium, P: phosphorus, creat: creatinine, Ptn: protein, FENa: fractional excretion of sodium, FEK: fractional excretion of potassium, TRP: tubular reabsorption of phosphorus, eGFR: estimated glomerular filtration rate.

[a] Significant $p < 0.05$.

[b] Independent t-test.

[c] Mann–Whitney test.

The urinary β2 microglobulin in all the studied patients was significantly and positively correlated to serum ferritin level as shown in Fig. 1 and transfusion index, ($r = 0.27$, $P = 0.04$ and $r = 0.32$, $P = 0.013$, respectively). The tubular reabsorption of P (TRP) was significantly higher among TM patients compared to TI patients ($P = 0.037$). A significant negative correlation was found between duration of chelation and TRP in TI patients ($P = 0.04$). Our study showed that the mean serum phosphorus level in all the study patients was within the normal range, but 20 (33.3%) of our cases; 18 (30%) TM patients and 2 (3.3%) TI patients had high serum phosphorus levels.

On the other hand, the mean serum ferritin level was significantly higher in TM than in TI patients ($P = 0.001$). There was a significant positive correlation between serum ferritin and transfusion index ($P = 0.001$). Also, a significant negative correlation existed between serum ferritin and mean pretransfusion hemoglobin ($P = 0.03$).

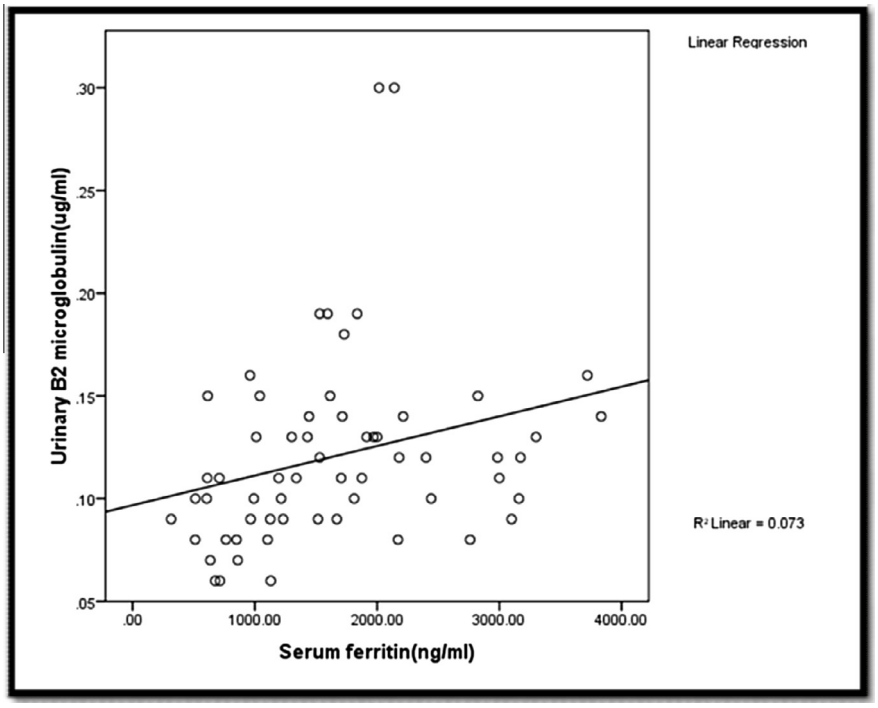

Figure 1 Shows a positive correlation between serum ferritin and urinary β2 microglobulin in all the study patients ($P = 0.04$).

5. Discussion

Several investigations about renal involvement in adult beta TM patients [14–16] were reported but there is a dearth of pediatric data. This study was designed to investigate the frequency of subclinical glomerular and tubular dysfunctions in children and adolescents with beta thalassemia major and intermedia.

22 of our patients were in the pubertal stage, of which 17 (77%) showed delayed puberty for age, 53% were males and 47% were females. Our results are in agreement with a previous study done by El-Beshlawy and Mokhtar on Egyptian β-thalassemia patients where failure of puberty was observed among 71.4% of males and 33.3% of females [17].

Our study showed a higher prevalence of splenectomy and hemosiderosis in thalassemia major patients compared to thalassemia intermedia, ($P = 0.91$ and $P = 0.03$), respectively. This might be due to higher serum ferritin levels among our TM patients when compared to TI patients. In contrast, Smolkin et al. mentioned that serum ferritin level does not sufficiently reflect the degree of hemosiderosis [18].

The mean level of serum creatinine in our patients was within the normal range. This is in consistence with other studies [7–10], where serum creatinine level was within the normal range among both TM and TI patients [19,20]. Other studies [21,22] reported that serum creatinine was within the normal range among TM patients; but it was higher when compared to that of the normal population (control group). In our study, serum creatinine level was comparable between TM and TI patients. Ali et al. [23] found that serum creatinine was significantly higher in TM than TI patients. Likewise, El-Alfyet et al. [24] reported that patients with thalassemia major had significantly higher serum creatinine and blood urea nitrogen values, possibly due to higher iron deposition in their kidneys. Our patients were on different types of chelation therapy and all cases proved to have normal serum creatinine. Previous studies reported normal serum creatinine in patients who received subcutaneous DFO treatment [8,25]. Other studies showed nephrotoxic effects of DFO in beta thalassemic patients [15]. The mean GFR in our study (done by Schwartz equation) was higher than the normal range in both TM and TI groups indicating glomerular hyperfiltration. This was in agreement with other studies [26], which found that a total of 24 thalassemia intermedia patients (48%) had evidence of glomerular hyperfiltration (>149 ml/min/1.73 m^2) for males and females. Other studies [9,18] however, found that the mean GFR (evaluated by Schwartz formula) was within the normal reference range (107.5 ± 1.27 ml/min/1.73 m^2), and was comparable in TM and TI patients. Glomerular hyperfiltration may be accounted for by the underlying anemia. There is evidence that anemia may reduce systemic vascular resistance, leading to a hyperdynamic circulation that can increase the renal plasma flow and GFR. Hyperfiltration can lead to stretching of the glomerular capillary wall and epithelial injury leading to progressive decline of GFR. Also when iron overload becomes severe it can cause damage of the tubular cells. In turn, the injured cells reverse into the interstitium cytokines and growth factors causing scarring and glomerular sclerosis [27].

As regards urinary protein/creatinine (U pr/Cr) ratio, the mean level of albumin/creatinine (A/C) ratio among our patients was 0.35 ± 0.81 mg/mmol which was higher than the normal range. Mohkam et al. [9] reported that 89.3% of their patients had abnormal U pr/Cr ratio. Also, Ziyadeh et al. [26] found that 30 of their patients (60%) had abnormal U pr/Cr ratio and it was positively correlated with serum ferritin ($P = 0.04$). In our study, unlike other studies there was no significant correlation between U pr/Cr ratio and serum ferritin ($P = 0.9$). Also, Ali and Mahmoud [28] found statistically higher ratios of A/C in urine of thalassemic children compared to the control group. This albuminuria was attributed mainly to the destruction of the glomerular filtration membrane which may be due to massive iron deposition in the tissues, resulting in an increase of free radical production via the Fenton reaction, leading to cell death by binding cell proteins and disturbing their production [29,30]. In addition, albuminuria could result from prolonged hyperfiltration, prostaglandin secretion and chronic anemia [31].

Urinary β2 microglobulin (β2MG) is a low molecular weight protein (11.8 kDa) which, under normal circumstances, is freely filtered at the glomerulus but almost totally reabsorbed and degraded by renal tubules [32]. Elevation of urinary β2MG is a sensitive and reliable early marker of tubular dysfunction [33,34]. In our study, the mean level of urinary β2 microglobulin was within the normal range (0.12 ± 0.05 μg/ml). Only 2 patients (3.3%) among TM group had slightly higher levels than normal while all TI patients included in our study showed normal urinary β2 microglobulin levels ($P = 0.01$). Similar results were reported in other studies where Mula-Abed et al. [35] reported that only 3(10%) of their patients had high urinary β2 microglobulin level. Meanwhile, earlier studies [15] reported that 13 of 19 TM patients had renal tubular damage, among them, 11 patients (85%) had high level of urinary β2 microglobulin. This marked difference among the compared studies may be due to improvement of thalassemic patients' care which probably led to decreased rate of proximal tubular damage.

Regarding tubular reabsorption of phosphorus (TRP), we found a significant difference between TM and TI patients included in our study. However, both were within the normal range. Similar findings were found by other studies [18,35], where no patients had TRP < 85%, which excluded the possibility of a renal tubular phosphate leak.

There was no affection in the mean fractional excretion of sodium (FENa)% and mean fractional excretion of potassium (FEK)% among our studied patients and no difference between both TM and TI patients groups denoting that there was no renal tubular affection regarding these mentioned parameters. This is in agreement with other studies [18]. However, Mohkam et al. [9], reported abnormal levels of FENa(%) and FEK(%) in 29.1% and 7.8% respectively among their studied patients. Mula-Abed et al. [35], found that almost all their studied patients had normal FENa(%) with only two patients (6.7%) showing slightly raised FENa > 1% indicating the possibility of developing prerenal failure which can be explained by prolonged anemia and hypovolemia which are constant findings in thalassemic patients.

The mean serum sodium and potassium among our cases were within the normal range. Similar findings were previously reported by other studies [8,14]. However, in our study there were significant differences between TM and TI in mean serum potassium ($P = 0.04$) and in mean serum sodium levels ($P = 0.01$). On the other hand, Al-Samarrai et al. [36] reported

higher levels of serum Na and K in thalassemic patients than the control group. An increase in serum Na level in TM may be due to renal damage resulting from iron overload in such patients, whereas, the increase in serum K level occurs in patients with RBCs hemolysis which may occur in stored blood that is transfused to patients since K tends to leak from RBCs to the plasma of stored blood [36].

Regarding serum ferritin in our study, the mean levels were 2044.88 ± 782.65 and 874.83 ± 379.88 ng/ml in TM and TI respectively, which showed a highly significant difference between the two groups ($P = 0.001$). This was similar to the study done by Alfery et al. [37] in which TM patients had a significantly higher mean serum ferritin level compared to TI patients; ($P < 0.05$). We also found a significant positive correlation between serum ferritin and transfusion index ($P = 0.001$) and a significant negative correlation between serum ferritin and mean pretransfusion hemoglobin ($P = 0.03$). This was in accordance with other studies [38], which found that increased number of blood transfusions were associated with increase in the serum ferritin level.

In our study, we further subdivided our TM patients according to the serum ferritin; into well chelated and poorly chelated groups where the mean serum ferritin level was significantly different between the two groups ($P = 0.001$). This was in agreement with other studies which reported similar results when they divided their patients according to their serum ferritin levels [38].

In our study, the mean height and height percentile in the poorly chelated group were lower than the well chelated group but the difference was not statistically significant. However, other studies [38] reported significant short stature for age among their poorly chelated group (serum ferritin $\geqslant 2500$ ng/ml) when compared to the well chelated group (serum ferritin < 2500 ng/ml) ($P < 0.001$). Pemde et al. [39], classified their thalassemic patients into two groups, one with serum ferritin < 2000 ng/ml and the other with serum ferritin > 2000 ng/ml, and they reported that the height of the patients with serum ferritin > 2000 ng/ml was significantly lower than those with serum ferritin < 2000 ng/ml. However, in an earlier study done in India by Gomber and Dewan [40], no relation between physical growth and serum ferritin level was found.

Notably, in the present study, we found that cases that underwent splenectomy were more prevalent among the poorly chelated group; 4 cases (40%) versus 10 cases (33.3%) among the well chelated group, while the difference was not statistically significant. Similar findings were reported by Bashir and Sadoon [38], where the number of splenectomized cases among the poorly chelated group (serum ferritin $\geqslant 2500$ ng/ml) was 5 (23.8%) compared to 9 (11.39%) in the well chelated group (serum ferritin < 2500 ng/ml) ($P < 0.001$).

As regards laboratory parameters of renal function, there was a significant difference between the mean serum sodium in the poorly chelated group and the well chelated group (134.45 ± 2.82 mmol/L vs. 131.22 ± 4.77 mmol/L), ($P = 0.01$). Also, the mean urinary protein/creatinine ratio in the poorly chelated group was significantly higher and above the normal range compared to the well chelated group (0.4 ± 0.42 mg/mmol vs. 0.23 ± 0.22 mg/mmol), ($P = 0.04$). This indicates a correlation between renal function in thalassemic patients and serum ferritin level. Other studies [18] have found a direct correlation of renal function disturbance to total amount of transfused iron but not to the actual serum ferritin level.

6. Conclusion

Subclinical renal affection is more common and could start earlier in TM patients compared to TI. Poor chelation is an important risk factor for early renal affection. Periodic renal assessment of those patients is mandatory as they may be affected by hidden renal dysfunction.

References

[1] Galanello R, Origa R. β-Thalassemia. Orphanet J Rare Dis 2010;5:11–25.

[2] Chen W, Zhang X, Shang X, et al. The molecular basis of β-thalassemia intermedia in southern China: genotypic heterogeneity and phenotypic diversity. BMC Med Genet 2010;11:31–40.

[3] Borgna-Pignatti C, Marsella M, Zanforlin N. The natural history of thalassemia intermedia. Ann N Y Acad Sci 2010;1202:214–20.

[4] Delecchio M, Cavallo L. Growth and endocrine function in thalassemia major in childhood and adolescence. J Endocrinol Invest 2010;33(1):61–8.

[5] Taher A, Isma'eel H, Cappellini MD. Thalassemia intermedia: revisited. Blood Cells Mol Dis 2006;37(1):2–12.

[6] Taher AT, Musallam KM, Karimi M, et al. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. Blood 2010;115(10):1886–92.

[7] Kattamis A. Treatment of thalassemia with hydroxyurea: an indispensable alternative therapy. J Pediatr Hematol Oncol 2007;29(11):729–30.

[8] Aldudak B, Karabay Bayazit A, Noyan A, et al. Renal function in pediatric patients with beta-thalassemia major. Pediatr Nephrol 2000;15:109–12.

[9] Mohkam M, Shamsian BS, Gharib A, et al. Early markers of renal dysfunction in patients with beta-thalassemia major. Pediatr Nephrol 2008;23:971–6.

[10] Koliakos G, Papachristou F, Koussi A, Perifanis V, Tsatra I, Souliou E, et al. Urine biochemical markers of early renal dysfunction are associated with iron overload in beta-thalassaemia. Clin Lab Haematol 2000;5(2):105–9.

[11] Giardina PJV, Forget BG. Thalassemia syndromes. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Silberstein LE, McGlave P, Heslop H, editors. Hematology: basic principles and practice. Philadelphia: Elsevier Churchill Livingstone; 2008.

[12] Thalassemia International Federation. Guidelines for the clinical management of thalassemia; 2008.

[13] Schwartz G, Brion L, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children and adolescents. Pediatr Clin North Am 1987;34:571–90.

[14] Sumboonnanonda A, Malasit P, Tanphaichitr VS, Ong-ajyooth S, Sunthornchart S, Pattanakitsakul S, et al. Renal tubular function in beta-thalassemia. Pediatr Nephrol 1998;12:280–3.

[15] Cianciulli P, Sollecito D, Sorrentino F, Forte L, Gilardi E, Massa A, et al. Early detection of nephrotoxic effects in thalassemic patients receiving desferrioxamine therapy. Kidney Int 1994;46:467–70.

[16] Michelakakis H, Dimitriou E, Georgakis H, Karabatsos F, Fragodimitri C, Saraphidou J, et al. Iron overload and urinary lysosomal enzyme levels in beta-thalassemia major. Eur J Pediatr 1997;156:602–4.

[17] El Beshlawy A, Mokhtar G, et al. Assessment of puberty in relation to L-carnitine and hormonal replacement therapy in beta-thalassemic patients. J Trop Pediatr 2008;54(6):375–81.

[18] Smolkin V, Halevy R, Levin C, et al. Renal function in children with beta-thalassemia major and thalassemia intermedia. Pediatr Nephrol 2008;23:1847–51.
[19] Ahmadzadeh A, Jalali A, Assar S, et al. Renal tubular dysfunction in pediatric patients with beta-thalassemia major. Saudi J Kidney Dis Transpl 2011;22:497–500.
[20] Quinn C, Johnson V, Kim H, et al. Renal dysfunction in patients with thalassemia. Brit J Hematol 2011;153:111–7.
[21] Hamed EA, El Melegy NT. Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study. Ital J Pediatr 2010:36–9.
[22] Grundy RG, Woods KA, Savage MO, et al. Relationship of endocrinopathy to iron chelation status in young patients with thalassaemia major. Arch Dis Child 1994;71:128–32.
[23] Ali D, Mehran K, Moghaddam AG, et al. Comparative evaluation of renal findings in Beta-thalassemia major and intermedia. Saudi J Kidney Dis Transpl 2008;19:206–9.
[24] El Alfy M, Sari TT, Chan LL, Tricta F, El-Beshlawy A. The safety, tolerability, and efficacy of a liquid formulation of deferiprone in young children with transfusional iron overload. J Pediatr Hematol Oncol 2010;32(8):601–5.
[25] Li Volti S, Di Gregorio F, Schiliro G, et al. Acute changes in renal function associated with deferoxamine therapy. Am J Dis Child 1990;144:1069–70.
[26] Ziyadeh F, Musallam K, Mallat N, et al. Glomerular hyperfiltration and proteinuria in transfusion-independent patients with beta-thalassemia intermedia. Nephr Clin Pract 2012;121:136–43.
[27] Ponticelli C, Musallam KM, Cianciulli P, et al. Renal complications in transfusion-dependent beta thalassaemia. Blood Rev 2010;24:239–44.
[28] Ali BA, Mahmoud AM. Frequency of glomerular dysfunction in children with beta thalassemia major. Sultan Qaboos Univ Med J 2014;14(1):e88–94, Epub: 27th Jan 14.
[29] Thomas CE, Morehouse LA, Aust SD. Ferritin and superoxide-dependent lipid peroxidation. J Biol Chem 1985;260:3275–80.
[30] Link G, Athias P, Grynberg A, Pinson A, Hershko C. Effect of iron loading on transmembrane potential, contraction, and automaticity of rat ventricular muscle cells in culture. J Lab Clin Med 1989;113:103–11.
[31] Koren G, Kochavi-Atiya Y, Bentur Y, Olivieri N. The effects of subcutaneous deferoxamine administration on renal function in thalassemia major. Int J Hematol 1991;54:371–5.
[32] Herrero-Morin JD, Malaga S, Fernandez N, Rey C, Diéguez MA, Solis G, et al. Cystatin C and beta-2-microglobulin: markers of glomerular filtration in critically ill children. Crit Care 2007;11:R59–66.
[33] Portman RJ, Kissane JM, Robson AM. Use of beta-2-microglobulin to diagnose tubulo-interstitial renal lesions in children. Kidney Int 1986;30:91–8.
[34] Guder WG, Hofmann W. Markers for the diagnosis and monitoring of renal tubular lesions. Clin Nephrol Suppl 1992;38:S3–7.
[35] Mula-Abed W, Al-Hashmi H, Al-Muslahi M. Indicators of renal glomerular and tubular functions in patients with beta thalassaemia major. SQU Med J 2011;11:69–76.
[36] Al-Samarrai AH, Adday MH, Khudhair A, et al. Evaluation of some essential element level in thalassemia major patients in Mousl district, Iraq. Saudi Med J 2008;29(1):94–7.
[37] Alfery AC. Role of iron and oxygen radicals in the progression of chronic renal failure. Am J Kidney Dis 1994;1(23):183–7.
[38] Bashir FY, Sadoon OA. Serum ferritin level in transfusion dependent beta thalassemia patients in Mousl. Ann Coll Med Mousl 2010;36(1&2):72–8.
[39] Pemde HK, Chandra J, Gupta D, et al. Physical growth in children with transfusion-dependent thalassemia. Dove Press J 2011;2:13–9.
[40] Gomber S, Dewan P. Physical growth patterns and dental caries in thalassemia. Ind J Pediatr 2006;43:1064–9.